

Serial No.: 09/633,274

i) first transcription unit comprising a lethal gene under control of a tapetum specific promoter and a transcription termination signal comprising a polyadenylation signal,

ii) second transcription unit comprising a selectable marker gene under control of a strong constitutive promoter and a transcription termination signal comprising a polyadenylation signal, and

iii) an insulator sequence which is about 5 kb in length, and which does not comprise transcriptional or other regulatory or enhancer elements, wherein the insulator sequence is placed between the first and second transcription units so as to isolate the first transcription unit from enhancing influences of the constitutive promoter in the second transcription unit,

wherein selection and use of the insulator sequence does not require prior knowledge of any inhibitor protein or any other regulatory component of the lethal gene, and

wherein the insulator sequence has the following properties:

(a) the insulator sequence does not encode any regulatory components or possess any enhancer elements or sequences that may influence the expression of neighboring genes;

(b) the insulator sequence has a GC content which is in consonance with transcriptionally active regions of a host genome;

(c) the insulator sequence does not produce any functional RNA or protein; and

Serial No.: 09/633,274

(d) the insulator sequence does not bear strict homology with any component of the host genome in order to avoid induction of homology dependent gene silencing.

2. (Amended) The construct as claimed in claim 1, wherein the lethal gene of the first transcription unit represents any coding sequence, which, upon expression in a plant cell, significantly disrupts the normal metabolism, function or development of the cell, thereby leading to death of the cell.

3. (Three Times Amended) The construct as claimed in claim 1, wherein the lethal gene is selected from the group consisting of *barnase*, *RnaseII*, *binase*, *rolB*, *rolC* and diphtheria toxin A.

4. (Three Times Amended) The construct as claimed in claim 1, wherein the lethal gene is *barnase*.

5. (Three Times Amended) The construct as claimed in claim 1, wherein the tapetum specific promoter of the first transcription unit is selected from the group consisting of TA29, A9, A3, *tap1* and *bcpl*.

6. (Twice Amended) The construct as claimed in claim 1, wherein the tapetum specific promoter is TA29.

7. (Three Times Amended) The construct as claimed in claim 1, wherein the selectable marker gene is a herbicide resistance-conferring gene selected from the group consisting of *bar*, *ALS*, and *tfdA*, or an antibiotic resistance-conferring gene selected from the group consisting of *nptII*, *hpt* and *aadA*.

8. (Three Times Amended) The construct as claimed in claim 1, wherein the selectable marker gene is *bar*.

9. (Twice Amended) The construct as claimed in claim 1, wherein the strong constitutive promoter is a CaMV35S promoter.

10. (Four Times Amended) The construct as claimed in claim 1, wherein the insulator sequence comprises coding sequences of a topoisomerase gene from pea and an acetolactate synthase gene from *Arabidopsis*.

13. (Twice Amended) A male sterile transgenic plant or seeds thereof, wherein the plant or seeds comprise in their nuclear genome the construct of claim 1.

Serial No.: 09/633,274

14. (Twice Amended) The plant as claimed in claim 13, wherein the plant is selected from the group consisting of a dicotyledonous and a monocotyledonous plant.

15. (Twice Amended) The plant of claim 13, wherein the plant is *Brassica juncea*.

16. (Four Times Amended) A method to obtain male-sterile plants in *Brassica juncea*, wherein said method comprises the steps of:

i) obtaining transformed plant cells by transforming the nuclear genome of plant cells with a foreign DNA comprising:

a) a first transcription unit comprising a lethal gene under control of a tapetum specific promoter and a transcription termination signal comprising a polyadenylation signal,

b) a second transcription unit comprising a selectable marker gene under control of a strong constitutive promoter and a transcription termination signal comprising a polyadenylation signal, and

c) an insulator sequence which is about 5 kb in length, and which does not comprise transcriptional or other regulatory or enhancer elements, wherein the insulator sequence is placed between the first and second transcription units, so as to isolate the first

transcription unit from enhancing influences of the constitutive promoter in the second transcription unit,

wherein the insulator sequence comprises the following properties:

(a) the insulator sequence does not encode any regulatory components or possess any enhancer elements or sequences that may influence the expression of neighboring genes;

(b) the insulator sequence has a GC content which is in consonance with transcriptionally active regions of a host genome;

(c) the insulator sequence does not produce any functional RNA or protein; and

(d) the insulator sequence does not bear strict homology with any component of the host genome in order to avoid induction of homology dependent gene silencing;

ii) regenerating male sterile transformed plants from the transformed plant cells,

iii) identifying male sterile transgenic plants from the regenerated plants of step (ii), wherein the male sterile plants exhibit normal vegetative morphology, normal female fertility, absence of pollen production and failure to set seed on selfing,

Serial No.: 09/633,274

iv) identifying the male sterile plants from step (iii) which have a single copy of the foreign DNA by Southern hybridization,

v) back-crossing the male sterile plants of step (iv) with an untransformed *Brassica juncea* plant to obtain a progeny population, and

vi) screening the progeny population of step (v) and selecting the male sterile plants that exhibit the characteristics of: normal seed germination frequencies, normal segregation ratio of marker gene and stable inheritance of male sterile phenotype.

23. (Three Times Amended) The method as claimed in claim 16, wherein the transformed plant cells of step (i) are generated by *Agrobacterium*-mediated transformation using disarmed Ti plasmid.

Please add the following claim:

--31. An insulator construct for controlling leaky expression of a lethal gene from enhancing functions of a strong constitutive promoter, wherein the insulator construct comprises:

i) first transcription unit comprising a lethal gene under control of a tapetum specific promoter and a transcription termination signal comprising a polyadenylation signal,

Serial No.: 09/633,274

ii) second transcription unit comprising a selectable marker gene under control of a strong constitutive promoter and a transcription termination signal comprising a polyadenylation signal, and

iii) an insulator sequence which is about 5 kb in length, and which does not comprise transcriptional or other regulatory or enhancer elements, wherein the insulator sequence is placed between the first and second transcription units so as to isolate the first transcription unit from enhancing influences of the constitutive promoter in the second transcription unit,

wherein the insulator sequence has the following properties:

(a) the insulator sequence does not encode any regulatory components or possess any enhancer elements or sequences that may influence the expression of neighboring genes;

(b) the insulator sequence has a GC content which is in consonance with transcriptionally active regions of a host genome;

(c) the insulator sequence does not produce any functional RNA or protein; and

(d) the insulator sequence does not bear strict homology with any component of the host genome in order to avoid induction of homology dependent gene silencing.--

**Attached hereto is a marked up version showing the changes made to the application by this Reply.**